AD-A101 133 INDIANA UNIV AT BLOOMINGTON DEPT OF CHEMISTRY F/G 7/4
A NEW INSTRUMENT FOR TIME-RESOLVED REDUCTION OF SCATTERED RADIA--ETC(U)
JUL 81 R E RUSSO, G M HIEFTJE N00014-76-C-0838
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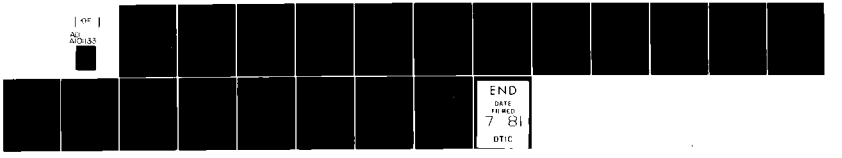

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1. REPORT NUMBER: THIRTY-NINE	2. GOVT ACCESSION NO.: AD-A101133
	3. RECIPIENT'S CATALOG NUMBER:
4. TITLE (and Subtitle): A New Instrument for Time-Resolved Reduction of Scattered Radiation in Fluorescence Measurements	5. TYPE OF REPORT & PERIOD COVERED: Interim Technical Report.
	6. PERFORMING ORG. REPORT NUMBER: 47. TR 3/47
7. AUTHOR(s): R. E. Russo and G. M. Hieftje	8. CONTRACT OR GRANT NUMBER(s): N14-76-C-0838
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Chemistry, Indiana University, Bloomington, IN 47405	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: NR 51-622
11. CONTROLLING OFFICE NAME AND ADDRESS: Office of Naval Research, Washington, D.C.	12. REPORT DATE: July 1, 1981
	13. NUMBER OF PAGES: 15
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office): LEVEL II	15. SECURITY CLASS. (of this report): UNCLASSIFIED
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE:

16. DISTRIBUTION STATEMENT (of this Report)

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

DTIC ELECTE JUL 7 1981 C

18. SUPPLEMENTARY NOTES

Prepared for publication in ANALYTICA CHIMICA ACTA

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Fluorescence
Time Resolution
Laser Spectroscopy
Correlation Spectroscopy

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

An opto-electronic cross-correlation system was employed to reduce the scattering influence in fluorescence measurements. A stable optical delay line incorporated into the instrument was positioned to yield detection at a fixed time after excitation; the optimal delay time was determined simply from the ratio of the fluorescence decay curve to a similar curve portraying scattering response. Signal-to-scattering background enhancements greater than two were measured for the very short-lived (τ = 0.07 ns) fluorophores whereas a six-fold increase was measured for longer lifetime fluorophores.

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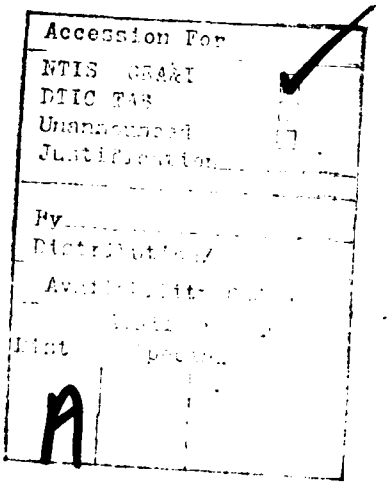
The shortest lifetime which would benefit from time-resolution in this system is limited by the time-response of the PMT (1.1 ns FWHM); the excitation pulses are on the order of 6 ps.



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Task No. NR 051-622

TECHNICAL REPORT NO. 39

A NEW INSTRUMENT FOR TIME-RESOLVED REDUCTION OF SCATTERED RADIATION IN FLUORESCENCE MEASUREMENTS

by

R. E. Russo and G. M. Hieftje

Prepared for Publication

in

ANALYTICA CHIMICA ACTA

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Bloomington, Indiana 47405

July 1, 1981

One of the main hinderances in fluorescence spectrometry is the scattering of excitation source radiation (1-4). For condensed-phase samples, not all of the light passes through a solution or is absorbed; some is scattered by finely dispersed particles, part is reflected from the cuvette surfaces (if not coated with an anti-reflection coating), and a fraction is Rayleigh scattered from the solvent (5). The effect of scattering is to deflect a portion of the exciting radiation into the fluorescence detection optical path, causing erroneously high measured intensities. Generally, scattered radiation is not directly detected in molecular fluorimetry because of a wavelength shift of the fluorescence from the absorption region; spectral discrimination is therefore possible. However, in low-resolution dispersing systems such as those employing filters, scattering can be detected directly. Moreover, even in higher quality systems, scattering contributes to stray light which can limit sensitivity, precision, and accuracy in molecular fluorimetry.

Scattering is potentially an even more serious problem in atomic fluorescence spectrometry (AFS) because resonance transitions are commonly employed. In AFS, scattering arises from refractive index inhomogeneities in the flame (Rayleigh scattering) and from incompletely atomized particles (Mie scattering); the greatest contribution is from Mie scattering (3,6). In fact, Mie scattering, which is the principal noise source in many atomic fluorescence measurements, limits accuracy, detection limits, and precision. Correction for or elimination of scattering is essential if atomic fluorescence spectrometry is to be used as a practical analytical

technique (7-11).

Several methods have been proposed for reducing the deleterious effects of scattering in AFS. Among these methods are:

a) use of non-resonance transitions - similar to molecular fluorimetry in that the fluorescence radiation is wavelength shifted from that of the incident radiation, and can be spectrally isolated. However, non-resonance transitions are less probable than resonance ones, and are not commonly employed in routine analysis (12,13),

b) polarization discrimination - unlike fluorescence, Rayleigh scattering exhibits a dependence on polarization and on direction of observation of the scattered light. By using linearly polarized light for excitation and by placing the detector at a right angle to the direction of the exciting light in the same plane as the polarization, one can minimize scatter while maintaining the fluorescence signal. However, this approach is effective only for reduction of Rayleigh scattering and is generally not applicable to flame atomic fluorescence where Mie scattering can be dominant (14),

c) wavelength modulation - scattering intensity is essentially constant over small changes in excitation wavelength, whereas atomic fluorescence is highly sensitive to wavelength shifts. Electro-optically tuned cw dye lasers can provide 1 nm repetitive wavelength scans at MHz modulation frequencies (14,16). Wavelength modulation and gated detection also preserve the

advantage of amplitude modulation in that background emission from the flame is minimized,

d) time-resolution - involves a pulsed excitation source and gated detection system. Scattering is essentially instantaneous and is therefore of the same duration as the excitation pulse, whereas fluorescence exhibits a finite lifetime. Therefore, the detection of scattered radiation can be greatly reduced if one measures fluorescence only after a finite time beyond excitation; i.e., after the excitation pulse is finished but before complete decay of the fluorescence radiation (17).

In the present work, this last method is explored. A variable, fixed-time delay spectrofluorimeter has been designed which is capable of eliminating most of the scattered radiation problem. Scatter is reduced by observing fluorescence a finite time after a light pulse excites the sample. This time-dependent observation is accomplished by means of a unique opto-electronic signal gating scheme constructed to perform the cross-correlation between two photodetector response functions (18-21). This arrangement is well suited for such studies because of the extremely narrow pulses provided by a synchronously pumped dye laser. In addition, an optical delay line is incorporated into the instrument and can be accurately positioned to measure fluorescence at any selected time after excitation, yet is free from triggering instability and drift.

Experimental

The experimental arrangement includes a synchronously pumped dye

laser and opto-electronic cross-correlation system described previously (18,21). Briefly, the pulses (6 ps FWHM) from a synchronously pumped dye laser (Model 171-06 Argon ion laser; Model 342 mode-locker with Model 452 mode-locker driver; Model 375 dye laser and Model 341 Synchronously Pumping Accessory Package, Spectra-Physics, Mountain View, CA) simultaneously irradiate a fast photodiode (Model 403B, Spectra-Physics) and sample cell (flame or cuvette) containing the fluorophore of interest. The fluorescence is detected by a fast response photomultiplier tube (Model 31024, RCA, Lancaster, PA), whose output is connected to one input of a double-balanced microwave mixer (Model ZFM-4, Mini-Circuits, New York, NY). The second input to the mixer receives the pulse from the fast photodiode, and the mixer performs a multiplication between the two signals. Because the photodiode signal is zero except when the laser strikes it, the signal serves as a "gate function" when it multiplies (in the mixer) the photomultiplier (PMT) output. Therefore, the photomultiplier output is sampled only during the time corresponding to the arrival of the laser pulse at the photodiode. Conveniently, the relative arrival time of the pulses at the two detectors (photodiode and photomultiplier) is easily varied by means of an optical delay line. Time-resolution of this delay line is 3 ps and its range of adjustment is approximately 12 ns. The delay is positioned so that the sample cell receives the laser pulse earlier than the photodiode. The time difference is chosen to be greater than the response time of the PMT under scattering conditions, but less than the lifetime of the fluorophore to be studied. When no fluorophore is present, the output of the mixer is approximately zero. However, when a fluorophore is

placed in the sample cell, the pulse width from the PMT is increased because of the finite lifetime of the fluorophore. Measurements of fluorescence intensity are therefore obtained in the absence of scattered radiation.

The actual procedure involves measuring the instrument response function (by directing scattered light toward the photomultiplier) and fluorescence decay curves (Figure 1) and determining from them the optimal position of the delay line. Specifically, the ratio of fluorescence intensity (S) to scatter intensity (B) was calculated as a function of optical delay setting. The delay time at which S/B is greatest is used to perform the scattering elimination experiments. Ideally, the fixed position of the delay line is such that sampling by the photodiode gate occurs in a temporal region where only fluorescence and no scattering exists (cf., Figure 1).

The influence of scatter was reduced in both atomic and molecular fluorescence measurements. Molecular fluorophores examined were Rhodamine B (τ= 2.79 ns) and Rose Bengal (τ= 0.70 ns), each of which was prepared at a concentration of 1 µM (in EtOH) with a fixed amount of polystyrene scattering spheres (0.22 ± 0.006 µm diameter, Dow Chemical Co., Indianapolis, In) added to the solution. Polystyrene sphere concentrations ranged from 0 to 40 µg/ml (6 solutions). Care was exercised in all measurements to ensure that the detected signal (fluorescence plus scatter) was within the linear range of the PMT response. Laser power was adjusted using a variable neutral-density filter to give the maximum permissible signal (below PMT saturation) when the fluorophore sample contained the highest concentration of scattering spheres.

In the measurement of atomic fluorescence, sodium (τ = 0.72 ns) at a concentration of 10 µg/ml (as NaCl solution) was mixed with four concentrations of $AlCl_3$: 100, 500, 1000, and 5000 ppm. Each solution was aspirated into a stoichiometric (F/O = 0.13) air-acetylene flame using a gas-dispersion-tube nebulizer. In this flame, Al is not significantly atomized, but is converted largely to desolvated particles which generate strong Mie scattering.

Results and Discussion

The relationship between time delay and the ratio of fluorescence (S) to scatter (B) is shown in Figure 2. The measured S/B ratio at zero-time delay is normalized to one; all measurements at successive time delays are related to this value. For the Rhodamine B solution with its relatively long lifetime (2.79 ns), a greater than six-fold increase in S/B is measured. However, for the shorter lifetime fluorophores Rose Bengal (0.70 ns) and Na (0.72 ns), the increase in S/B is not as great, but is still significant. Any such increase in S/B is valuable in that detection limits can be lowered and the precision of fluorescence measurements increased (2).

From the curves in Figure 2, one can determine the optimum (best S/B) position of the optical delay line for the elimination of scatter. For Rhodamine B, a delay of 3 ns was chosen, whereas a 1.6 ns delay was employed for both Rose Bengal and Na measurements.

The degree to which scatter can be eliminated through time-resolution is revealed in Figure 3, where the total measured signal (ostensibly from fluorescence alone) is plotted versus the concentration of scattering spheres. In conventional steady-state

fluorimetry, the presence of a scattering substance in the fluorophore solution would cause erroneously high intensity levels (Figure 3A). Of course, with a high-resolution spectrometer, little scattering would be detected directly and curve A would exhibit a lower slope. However, in the present measurement system, a broad-band (10 nm) interference filter was employed to effect the worst case.

The strong scatter/stray light signal in curve A is greatly reduced by measuring the fluorescence intensity at a finite delay after excitation (Figure 3, B and C). As expected (cf., Fig. 2), the longer this delay time, the better the rejection of the scattering interference. The slight decrease in measured signal with scatterer concentration (cf., Fig. 3C) can be attributed to a reduction in source intensity in the observed sample volume caused by scattering-induced losses. This effect is similar to the inner-filter behavior (13) common in molecular fluorimetry. Specifically, as the concentration of scattering spheres is increased, the intensity of the exciting laser beam is attenuated by scattering losses as it traverses the sample cell. Concurrently, the fluorescence itself is scattered during its passage from the laser-beam location in the cell toward the detection system. For the relatively short lifetime of excited state Rose Bengal in solution, the measured increase in relative fluorescence intensity results from an offset of this inner-filter effect by a relatively high scatter/stray signal. For this solution, the optical delay time (cf., Fig. 2) is so close to the scatter (instrument) response curve that a significant amount of scatter can still be detected. A longer delay time could be employed if further scattering reduction is necessary; however, a significant loss of

fluorescence signal would result. It is expected that Figure 3B would exhibit an even greater slope (more closely follow the zero-time delay scatter - Fig. 3A) if inner-filter effects were absent.

Freedom from the effects of scattered radiation in Na atomic fluorescence is displayed in curve B of Figure 4, where a 1.6 ns time delay was employed between excitation and detection. In this experiment, added $AlCl_3$ increased the density of scattering sites in the flame, as seen in curve A. However, the use of time-delayed detection (curve B) eliminates most of the scattering error. There are at least three possible competing processes that affect the measured fluorescence signal in Figure 3: inner-filter effects from scattering by incompletely atomized Al particles; occlusion of the Na by Al in solution, and detected scattered/stray light intensity. However, occlusion was found to be negligible in a separate experiment; equal emission intensity was measured for Na with 0 and 5000 ppm Al in solution. Similar to the Rose Bengal case (cf., Fig. 3), detection of scatter/stray light is expected at the 1.6 ns delay because the fluorescence decay overlaps the background (scatter) pulse.

Acknowledgement

Taken in part from the PhD thesis of R. E. Russo. Supported in part by the National Institute of Health through grant PHS GM 24473, by the Office of Naval Research and the the National Science Foundation.

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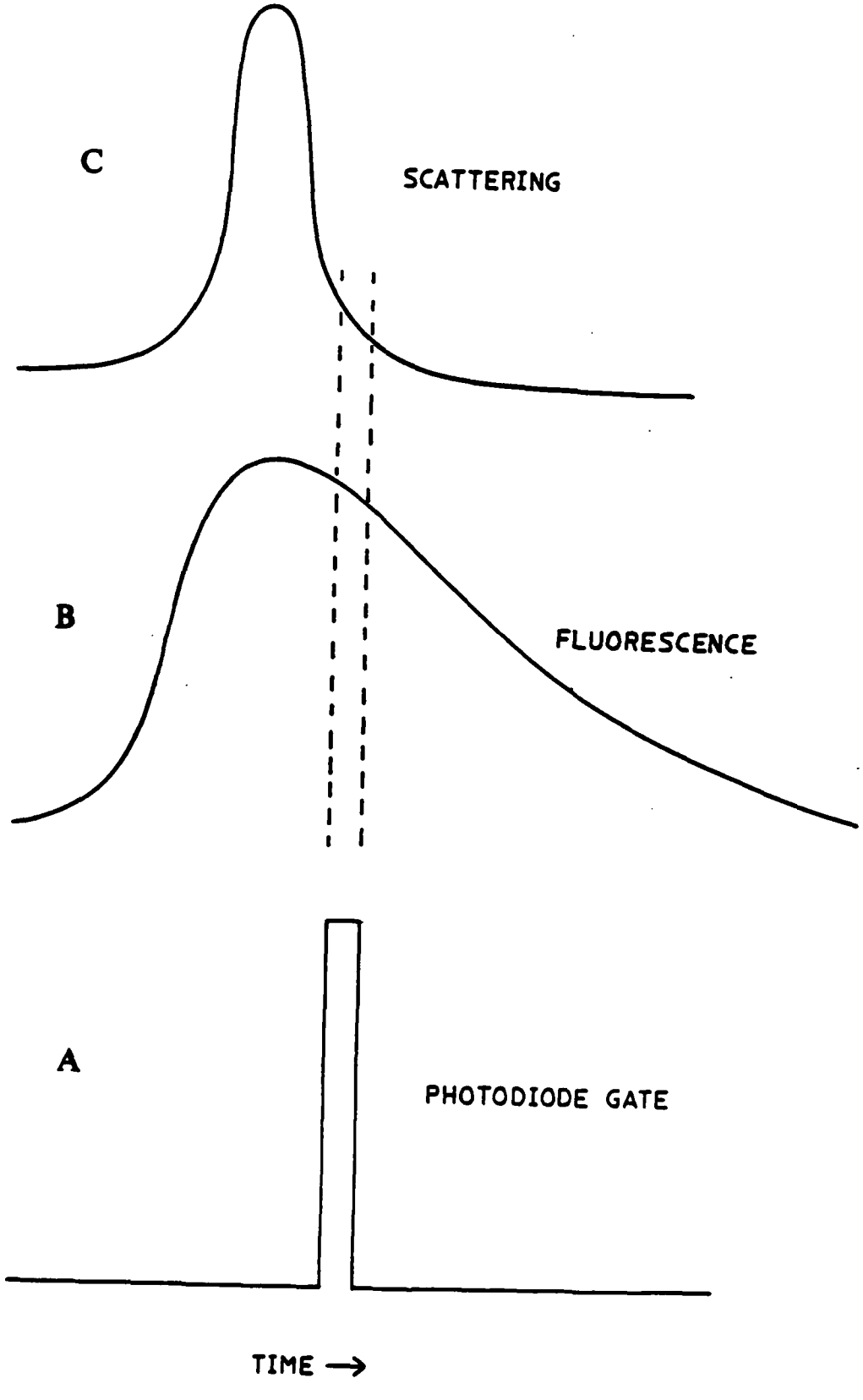
Figure Captions

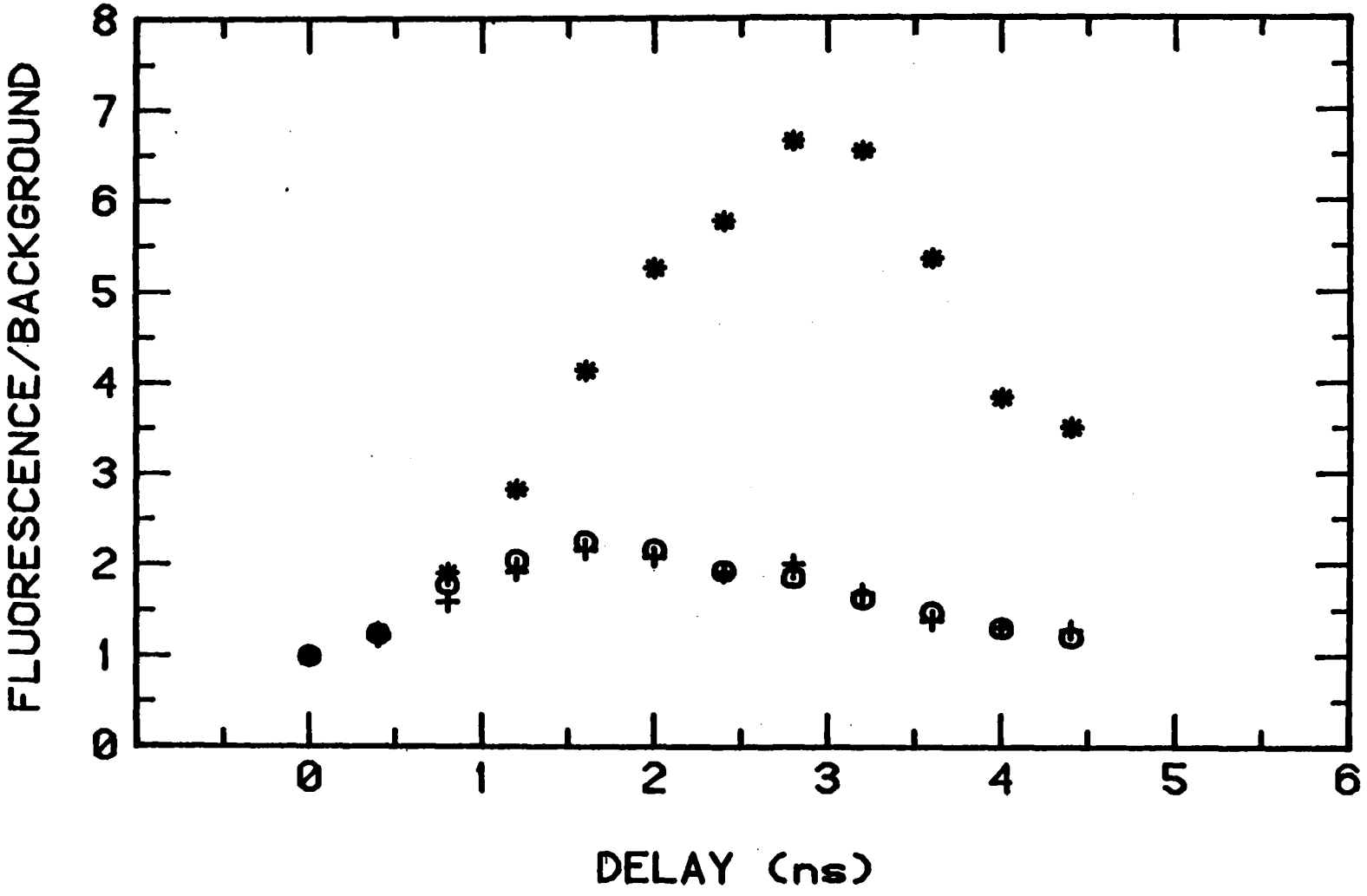
Figure 1. Diagram showing ideal position of sampling gate (A) with respect to the fluorescence response (B) and impulse response (C) curves.

Figure 2. Signal-to-background enhancement with time-delay sampling. * Rhodamine B, τ= 2.79 ns; 0 Rose Bengal, τ= 0.70 ns; and + Na, τ= 0.72 ns.

Figure 3. Reduction of scatter by time resolution. A, Rhodamine B (τ= 2.79 ns), delay line at 0.0 ns (conventional); B, Rose Bengal (τ= 0.70 ns), delay line at 1.6 ns; and C, Rhodamine (τ= 2.79 ns), delay line at 3.0 ns.

Figure 4. Reduction of scattered radiation for Na atomic fluorescence in an air-acetylene flame. A, conventional sampling, no time delay after excitation; and B, sampling 1.6 ns after excitation.


C
SCATTERING
B
FLUORESCENCE
A
PHOTODIODE GATE
TIME →


FLUORESCENCE/BACKGROUND
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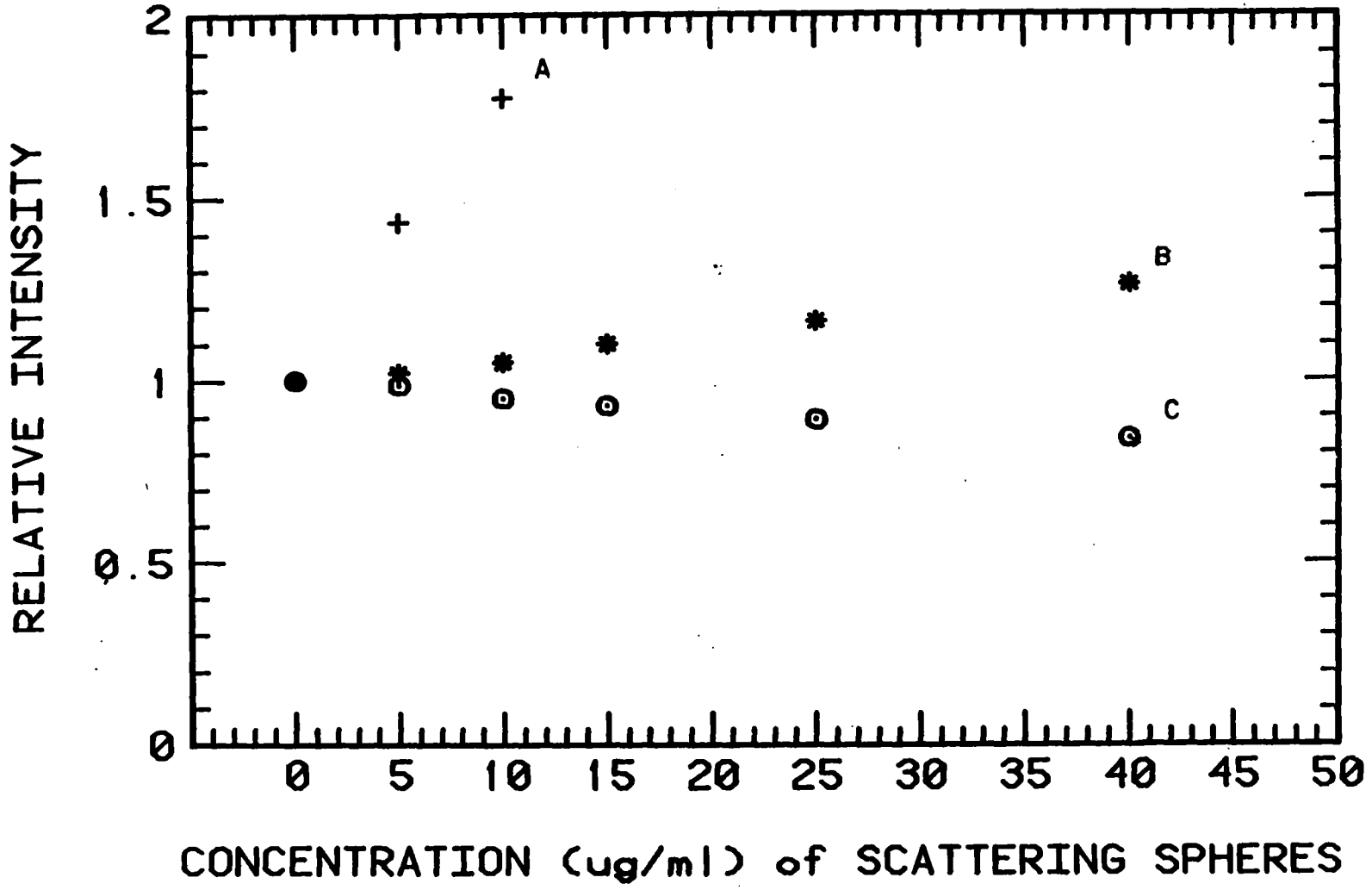
DELAY (ns)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1
0.5
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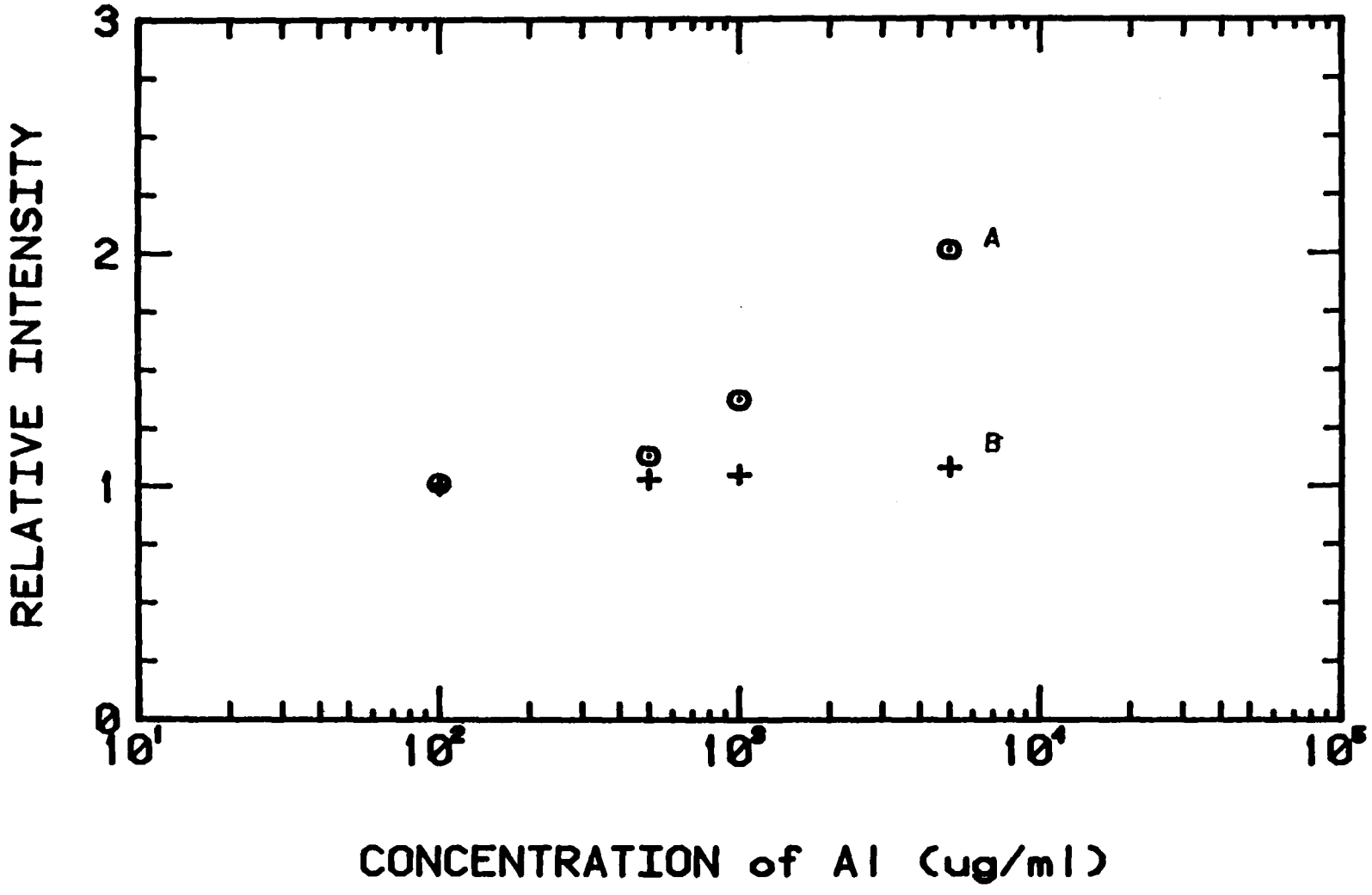
50
CONCENTRATION (ug/ml) of SCATTERING SPHERES


RELATIVE INTENSITY
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10²
10³
10⁴
10⁵
CONCENTRATION of Al (ug/ml)

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